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THE EXPLANATION OF THE COLLOIDAL BEHAVIOR OF PROTEINS[1]

I

THIS year's Pasteur lecture coincides with the commemoration of the hundredth anniversary of Pasteur's birth. The application of Pasteur's ideas and discoveries has benefited humanity to such an extent that they have become part of the consciousness of civilized mankind. What is, perhaps, less widely understood is the fact that Pasteur changed the method of medical research. In the study of infectious diseases Pasteur substituted for the method of hit or miss (with the chances infinitely in favor of missing) the method of a definitely oriented search which never fails to give results when properly applied. Thousands of physicians had studied infectious diseases before Pasteur, but they tried to solve their problem by starting from observations of the symptoms of some special disease. This led to no result for the simple reason that without knowing beforehand for what to look—or, in other words, without knowing the general cause of infectious diseases—it was impossible to discover the cause of any special infectious process. Pasteur reversed this method by his discovery of the action and omnipresence of microorganisms, leaving it to the medical men to look for the special agency in the individual cases.

There is little doubt that the old empiricism, still in vogue in some other fields of medicine and in the physiological sciences, must be replaced by the more rationalistic method of Pasteur of knowing the general fundamental principles before attempting to explain the more special phenomena, since, unless we follow this method, we never know which of

[1] Pasteur Lecture delivered before the Institute of Medicine of Chicago on November 24, 1922.

the details we observe are significant and which are negligible.

II

Living matter is essentially colloidal in character and we can not well conceive of an organism consisting exclusively of crystalloidal matter. This fact suggests that life phenomena depend upon or are intrinsically linked with certain characteristics of colloidal behavior. It is, therefore, natural that a systematic study of the nature of special life phenomena should be preceded by a scientific theory of colloidal behavior. By a scientific theory, however, we do not understand speculations or guesses built on qualitative experiments or no experiments at all, but the derivation of the results from a rationalistic, mathematical formula which permits us to calculate, with an adequate degree of accuracy, the quantitative measurements of colloidal behavior.

Proteins are amphoteric electrolytes which are capable of forming salts with either alkalies or acids. With alkalies they form salts like Na proteinate, Ca proteinate, etc., and with acids they form salts like protein chloride, protein sulfate, etc. Whether they do the one or the other depends on the hydrogen ion concentration of the protein solution. There is one definite hydrogen ion concentration at which a protein can combine practically with neither acid nor alkali, and this hydrogen ion concentration, which may be different for different proteins, is called the isoelectric point. The isoelectric point is (in terms of Sörensen's logarithmic symbol) for gelatin and casein at p_H 4.7; for crystalline egg albumin at p_H 4.8. Gelatin can combine with acid only or practically only when the p_H is less than 4.7 and with alkali only or practically only when the p_H is higher than 4.7. Or in other words, when a salt, *e. g.*, $NiCl_2$, is added to gelatin solutions of different p_H, Ni gelatinate can only be formed when the p_H is greater than 4.7; and when $K_4Fe(CN)_6$ is added gelatin-$Fe(CN)_6$ can only be formed when the p_H is less than 4.7. This can be shown by methods discussed in a recent book.[2]

2 Loeb, J.: "Proteins and the Theory of Colloidal Behavior," New York and London, 1922.

The proof that proteins combine stoichiometrically with acids and alkalies can be furnished by titration curves. For this purpose (and perhaps for work with proteins in general) it is necessary to use as standard material protein of the p_H of the isoelectric point. We have seen that proteins combine with acids only at a p_H below that of the isoelectric point, which for gelatin or casein is about p_H 4.7 and for crystalline egg albumin 4.8. It happens that at a p_H below 4.7 most of the weak dibasic and tribasic acids dissociate as monobasic acids. Thus H_3PO_4 dissociates into H^+ and the monovalent anion $H_2PO_4^-$ Hence if acids combine stoichiometrically with isoelectric protein, it should require exactly three times as many cc. of 0.1 N H_3PO_4 to bring a 1 per cent. solution of an isoelectric protein, *e. g.*, gelatin or crystalline egg albumin or casein, to the same hydrogen ion concentration, *e. g.*, p_H 3.0, as it requires of 0.1 N HCl or HNO_3. Titration experiments show that this is the case. Furthermore, since H_2SO_4 is a strong acid, splitting off both hydrogen ions even at a p_H below 4.7, the same number of cc. of 0.1 N H_2SO_4 as of HCl should be required to bring 1 gm. of isoelectric protein in 100 cc. of solution to the same p_H, *e. g.*, 3.0, and this was found also to be true.

Fig. 1 gives the titration curves for crystalline egg albumin for four acids, HCl, H_2SO_4, H_3PO_4, and oxalic acid. One gram of isoelectric albumin was in 100 cc. H_2O containing

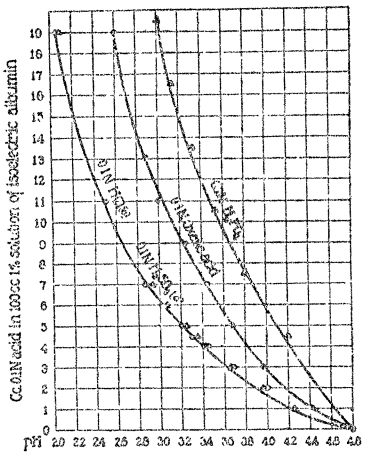

FIG. 1

various cc. of 0.1 N acid. These cc. of 0.1 N acid in 100 cc. solution are the ordinates of the curves in Fig. 1. The abscissæ are the p_H to which the protein solution was brought by the addition of acid. It takes always exactly three times as many cc. of 0.1 N H_3PO_4 as it takes cc. of 0.1 N HCl or H_2SO_4 to bring 1 gm. of isoelectric albumin in 100 cc. solution to the same p_H. In order to bring the 1 per cent. solution of originally isoelectric albumin to p_H 3.2, 5 cc. of 0.1 N HCl or H_2SO_4 and 15 cc. of 0.1 N H_3PO_4 must be contained in 100 cc. of the solution. To bring the albumin to p_H 3.4, 4 cc. of 0.1 N HCl or H_2SO_4 and 12 cc. of 0.1 N H_3PO_4 must be contained in the solution, and so on.

Oxalic acid is, according to Hildebrand, a monobasic acid at a p_H of 3.0 or below, but begins to split off the second hydrogen ion in increasing proportion above p_H 3.0. The titration curves show that about twice as many cc. of 0.1 N oxalic acid as 0.1 N HCl are required to bring the 1 per cent. solution of isoelectric albumin to the same p_H below 3.0, while it takes less than twice as many cc. of 0.1 N oxalic acid as 0.1 N HCl to bring the albumin solution to the same p_H if the p_H is above 3.0.

It can be shown in the same way with the aid of titration curves that isoelectric albumin combines with alkalies in the same stoichiometrical way as any acid, *e. g.*, acetic acid, would combine with the same alkalies. If the cc. of 0.1 N KOH, NaOH, $Ca(OH)_2$, or $Ba(OH)_2$ in 100 cc. solution required to bring a 1 per cent. solution of isoelectric protein to the same p_H are plotted as ordinates over the p_H of the protein solution as abscissæ, it is found that the values for all four alkalies fall on one curve as they should if the combination occurred strictly stoichiometrically.

The same stoichiometrical results were obtained also with casein and gelatin by the writer, and with edestin and serum globulin by Hitchcock. There is little doubt that they will be obtained in the case of all proteins. It follows from this that proteins react with acids and alkalies in the same way as do amphoteric crystalloids like amino-acids. If the methods for measuring the hydrogen ion concentrations of protein solutions had been employed by the colloid chemists nobody would have thought of suggesting that proteins react with acids and alkalies according to the empirical adsorption formula of Freundlich instead of stoichiometrically.

The purely chemical character of the combination of proteins with hydrochloric acid can also be demonstrated by measuring the chlorine ion concentration of the solutions of protein chloride. When HCl is added to NH_3 (according to Werner) the H ions of the HCl are attracted to the nitrogen of the ammonia, while the Cl ions remain unaltered. The same type of reaction occurs when HCl is added to a solution of isoelectric gelatin. This was proven by measurements of the p_{Cl} of solutions of gelatin chloride. Different cc. of 0.1 N HCl were contained in 100 cc. of 1 per cent. solutions of originally isoelectric gelatin and the p_H and p_{Cl} of the solutions were measured, the p_H with the hydrogen electrode and the p_{Cl} with the calomel electrode. It was found that the p_{Cl} was the same as if no gelatin had been present while the p_H was, of course, higher; thus showing that part of the hydrogen combines with the NH_2 and NH groups of the protein molecule while the Cl remains free (Table I). Dr. Hitchcock has obtained similar results with crystalline egg albumin, edestin, casein, and serum globulin, by using a silver chloride electrode, so that it is possible to state that these results are true for most if not all proteins.

TABLE I

Cubic centimeters of 0.1 N HCl in 100 cc. solution	Solution containing no gelatin		Solution containing 1 gm. of isoelectric gelatin in 100 cc.	
	p_H	p_{Cl}	p_H	p_{Cl}
2	2.72	2.72	4.2	2.68
3	2.52	2.54	4.0	2.53
4	2.41	2.39	.....	.....
5	2.31	2.29	3.60	2.33
6	2.24	2.26	3.41	2.25
7	2.16	2.18	3.23	2.18
8	2.11	2.12	3.07	2.11
10	2.01	2.01	2.78	2.025
15	1.85	1.85	2.30	1.845
20	1.72	1.76	2.06	1.76
30	1.55	1.59	1.78	1.60
40	1.43	1.47	1.61	1.47

The titration curves prove another fact, namely, that the salts of proteins are strongly hydrolyzed. When we add acid, *e. g.*, HCl, to isoelectric protein, part of the acid combines with the protein giving rise to protein chloride, while the rest of the acid remains free. There is then an equilibrium between free HCl, protein chloride, and non-ionogenic (or isoelectric) protein. The more acid is added to originally isoelectric protein, the more protein chloride is formed until finally all the protein exists in the form of protein chloride. It is possible to find out from the p_H measurements how much of the acid added is free and by deducting this value we know how much is in combination with the protein. By saturating the protein with acid the combining weight of a protein with acid can be found. Hitchcock found in this way that the combining weight of gelatin is about 1090.

III

The colloidal behavior of proteins shows itself in a peculiar effect of electrolytes—acids, alkalies or salts—on such properties as the swelling of gels or the osmotic pressure or viscosity of protein solutions. All these properties, swelling, osmotic pressure, viscosity, are affected by electrolytes in a very similar way; suggesting that all are due to the same cause. We shall see that by giving the explanation for one of these properties, osmotic pressure, we shall by implication give the explanation for all of them.

Measurements of the osmotic pressure of solutions of a protein—gelatin, crystalline egg albumin, casein and edestin—were made with solutions containing 1 gm. dry weight of originally isoelectric protein in 100 cc. of solution; and the 100 cc. of solution included also varying concentrations of 0.1 N acid. These solutions were put into collodion bags suspended in water free from protein. The outside water was at the beginning of the experiment brought to the same p_H as that of the protein solution, using always the same acid as that added to the protein. The measurements of the osmotic pressure were read after 18 hours when osmotic equilibrium was established. It was found that the osmotic pressure varied in a characteristic way with the p_H of the protein solution and the valency of the anion of the acid used. This effect is shown in the curves in Fig. 2 which were obtained from gelatin solutions. But the curves are similar in the case of other proteins such as crystalline egg albumin, casein or edestin. These curves show that the osmotic pressure of a protein solution is a minimum at the isoelectric point, that it increases when little acid is added until a maximum is reached, and that on the further addition of acid the osmotic pressure is again diminished. They show, moreover, that only the valency and not the nature of the anion of the acid influences the osmotic pressure of a protein solution. We know from the titration curves that in the case of H_3PO_4 the anion in combination with the protein is not the trivalent PO_4 but the monovalent H_2PO_4; and the curves in Fig. 2 show that the influence of phosphoric acid and hydrochloric acid on the osmotic pressure is the same if measured for the same p_H of the protein solution. Oxalic acid is a monobasic acid below p_H 3.0 and we notice that the descending branch of the oxalic acid curve below p_H 3.0 practically coincides with the descending branch of the HCl curve. The curve for the influence of H_2SO_4 is only about half as high as that for HCl and we know from the titration curves that the anion of protein sulfate is bivalent. It was found that all monobasic acids, *e. g.*, HBr, HNO_3, acetic acid, etc., and all weak dibasic or tribasic acids, *e. g.*,

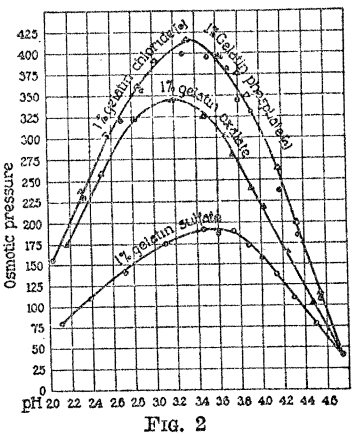

FIG. 2

tartaric, malic, citric, etc., which below p_H 4.7 dissociate as monobasic acids, give osmotic pressure curves identical with those for HCl and H_3PO_4. We may, therefore, draw the conclusion that only the valency but not the nature of the acid influences the osmotic pressure of protein solutions, that all acids which are monobasic on the acid side of the isoelectric point of a protein influence its osmotic pressure in the same way, and that this influence is considerably greater than the influence of strong dibasic acids like H_2SO_4.

If alkali is added to a solution of isoelectric protein it can be shown that the addition of little alkali increases the osmotic pressure until a maximum is reached when the addition of more alkali depresses the osmotic pressure again. All alkalies with monobasic cation like Li, Na, K, NH_4, have the same effect at the same p_H, while alkalies and all dibasic cations like Ca or Ba act alike, the curve for the effect of the alkalies with divalent cation being only about half as high as that of the alkalies with monovalent cation.

A third fact (discovered by R. S. Lillie) is that the addition of salts to a solution of a protein salt always depresses the osmotic pressure.

The curves representing the influence of acids and salts on the osmotic pressure are almost identical or very similar to those representing the influence of the same acids and salts on swelling and viscosity. These results are specific for colloidal behavior and any theory of colloidal behavior will have to give not only a qualitative but a quantitative theory of these curves.

It was suggested by Zsigmondy that the influence of acid on osmotic pressure was due to an influence on the degree of dispersion of the protein in solution, but since the degree of dispersion can not be accurately measured, this suggestion is only a qualitative speculation. But it is of no use even as a qualitative speculation since it fails to account for the fact that viscosity and swelling are affected in a similar way as osmotic pressure. The correct explanation is as follows: When acid (or alkali) is added to a solution of an isoelectric protein, part or all of this is transformed into an ionizable protein salt according to the amount of acid added. This ionization of the protein causes the colloidal behavior on account of the inability of protein ions to diffuse through membranes which are easily permeable to crystalloidal ions, such as collodion or parchment membranes or the walls of capillaries or probably of all cells. Now it was shown by Donnan that whenever the diffusion of one type of ions such as colloidal ions is prevented by a membrane which is readily permeable to crystalloidal ions, an unequal distribution of the diffusible crystalloidal ions results on the opposite sides of the membrane. This unequal distribution of diffusible crystalloidal ions is the cause of the colloidal behavior of proteins.

IV

When a collodion bag is filled with a solution of gelatin chloride of p_H 3.0 and the bag is immersed in an aqueous solution of HCl also of p_H 3.0 but free from protein, acid is driven from the protein solution into the outside aqueous solution free from protein. Donnan has shown thermodynamically that when osmotic equilibrium is established the products of the concentrations of each pair of oppositely charged diffusible ions (*e. g.*, H and Cl in the case of gelatin chlroide) are equal on the opposite sides of the membrane. Let x be the molar concentration of the H and Cl ions on the outside, y the molar concentration of the free H and Cl ions inside the protein solution, and z the concentration of the Cl ions in combination with the protein; then equilibrium is defined by the following equation, first used by Procter and Wilson to explain the influence of acid on swelling,

$$x^2 = y(y + z) \qquad (1)$$

The first step in an attempt to explain the influence of acids, alkalies and salts on the osmotic pressure of protein solutions is to find out whether the variations in osmotic pressure under the influence of acids as shown in Fig. 2 are accompanied by corresponding differences in the concentration of diffusible ions inside and outside the protein solution and whether these differences can be calculated from Donnan's equilibrium equation (1).

The writer was able to show that this is true by making measurements of a property of protein solutions, which had received little if any attention in colloid chemistry, namely, the measurements of the membrane potentials existing between a protein solution and the surrounding aqueous solution at the time of osmotic equilibrium.

Donnan's equilibrium formula can be written in the form

$$\frac{x}{y} = \frac{y + z}{x}$$

where $\frac{x}{y}$ is the ratio of the molar concentration of the hydrogen ions outside to the concentration of the hydrogen ions inside, while $\frac{y + z}{x}$ is the ratio of the molar concentration of the chlorine ions inside to that outside. Donnan had shown that there should exist a potential difference between the inside and outside solutions, which at 24° C. should be equal to $59 \times \log \frac{x}{y}$ millivolts or $59 \times \log \frac{y + z}{x}$ millivolts. Since p_H inside is $= -\log y$ and p_H outside is $= -\log x$, $\log \frac{x}{y}$ is equal to p_H inside minus p_H outside. p_H inside and p_H outside can be determined directly with the aid of the hydrogen electrode; $\log \frac{y + z}{x}$ is equal to p_{Cl} outside minus p_{Cl} inside and this quantity can be measured directly by titration or with the silver chloride electrode.

On the other hand, the P.D. between the protein solution and the surrounding aqueous solution across a collodion membrane can be measured directly with the aid of a Compton electrometer and a pair of identical indifferent calomel electrodes (and saturated KCl). If the unequal distribution of diffusible crystalloidal ions (*e. g.*, H and Cl in the case of gelatin chloride) on the opposite sides of the membrane is really determined by the Donnan equilibrium, then the P.D. observed directly with the pair of identical calomel electrodes should be equal to the P.D. calculated in millivolts from the values $59 \times$ (p_H inside minus p_H outside) or from $59 \times$ (p_{Cl} outside minus p_{Cl} inside), where p_{Cl} or p_H may be obtained by titration or by the silver chloride or hydrogen electrodes respectively. The writer has made these measuremeents and found that when various quantities of acid are added to solutions of isoelectric protein—*e. g.*, crystalline egg albumin, or gelatin, or casein—the observed membrane potentials always agree with the membrane potentials calculated on the basis of Donnan's equation within one or two millivolts, *i. e.*, within the limits of accuracy of the measurements.

The net result of extensive measurements of membrane potentials was, first, that when a protein solution, enclosed in a collodion bag (impermeable to protein ions but permeable to crystalloidal ions), is in osmotic equilibrium with an outside aqueous solution, the concentrations of crystalloidal ions in the protein solution and in the outside aqueous solution are not the same; and second, that the difference in the two concentrations can be calculated from Donnan's equilibrium equation.

V

We are now in a position to explain the osmotic pressure curves in Fig. 2. The colloid chemists would have taken it for granted that such curves were due to an influence of the acids on the state of dispersion or on some other real or imaginary colloidal property of proteins. Before we have a right to indulge in such speculations we must realize that these curves of observed osmotic pressure are not exclusively the expression of the osmotic pressure due to the protein particles, or protein molecules, and protein ions alone, but are also the result of the demonstrable unequal concentrations of the crystalloidal ions on the opposite sides of the membrane, caused by the establishment of a Donnan equilibrium. In other words, the observed osmotic pressure of a protein solution needs a correction due to the Donnan equilibrium before we can begin to speculate on the cause of the influence of acid on these curves, and it is our purpose to calculate the value of this correction.

We begin with the curve expressing the influence of HCl on the osmotic pressure of a 1 per

cent. solution of originally isoelectric gelatin and we consider the distribution of ions inside the protein solution and in the aqueous solution outside the collodion bag containing the protein solution at osmotic equilibrium. We also assume complete electrolytic dissociation of gelatin chloride as well as HCl. Let a be the molar concentration of the protein molecules and ions, let z be the molar concentration of the Cl ions in combination with the ionized protein, let y be the molar concentration of the hydrogen ions of the free HCl inside the protein solution; the molar concentration of the Cl ions of this HCl is also y. In that case the osmotic pressure of the protein solution is determined by

$$a + 2y + z$$

From this must be deducted the osmotic pressure of the HCl of the outside aqueous solution. If x is the molar concentration of the H ions of the outside solution, it is also the molar concentration of the Cl ions. Hence the observed osmotic pressure of a protein solution is determined by the following molar concentration,

$$a + 2y + z - 2x$$

Fig. 2 shows how this value varies with the p_H of the protein solution (*i. e.*, y). In order to arrive at a theory concerning the influence of HCl on the osmotic pressure of protein solutions it is necessary to calculate the value of $2y + z - 2x$ and to deduct it from the observed osmotic pressure of the protein solution. The term $2y + z - 2x$ we will call the Donnan correction. In this term y and x can be calculated from the measurements of the p_H, p_H inside being $-\log y$ and p_H outside being $-\log x$. z can be calculated from x and y with the aid of the Donnan equation (1)

$$z = \frac{(x + y)(x - y)}{y}$$

since we now know that x and y are determined by the Donnan equilibrium. If the value of $2y + z - 2x$ is calculated for different p_H of a gelatin chloride solution (of the same concentration of originally isoelectric gelatin which in this case was 1 per cent.); and if from this value is calculated the osmotic pressure due to this excess of the molar concentration of crystalloidal ions inside the protein solution over that outside, it is found that the curve for the Donnan correction is almost identical with the curve for the observed osmotic pressure. In other words, it turns out that the increase in osmotic pressure of a 1 per cent. solution of originaly isoelectric gelatin upon the addition of little acid until a maximum is reached, and the diminution of osmotic pressure upon the addition of further acid are not due to any variation in the state of dispersion of the protein, or any other real or imaginary "colloidal" property of the protein, but purely to the fact that protein ions can not diffuse through the collodion membrane which is easily permeable to crystalloidal ions; as a consequence of which the molar concentration of the crystalloidal ions must always be greater inside the protein solution than outside. What varies with the p_H of the gelatin solution is the quantity of the excess of $2y + z$ over $2x$. This follows from the Donnan equation (1) according to which

$$x = \sqrt{y^2 + yz} \text{ or } 2x = \sqrt{4y^2 + 4yz}$$

while

$$2y + z = \sqrt{4y^2 + 4yz + z^2}$$

Now it is obvious that

$$\sqrt{4y^2 + 4yz + z^2} > \sqrt{4y^2 + 4yz}$$

i. e., the concentration of the crystalloidal ions inside the protein solution $2y + z$ is always greater than the concentration of the crystalloidal ions $2x$ outside, when z is not 0 or ∞.

If we substitute for the term $2y + z - 2x$ of the Donnan correction the identical term

$$\sqrt{4y^2 + 4yz + z^2} - \sqrt{4y^2 + 4yz}$$

we can visualize why the osmotic pressure is a minimum at the isoelectric point, why it increases with the addition of little acid, reaching a maximum, and why it diminishes again with the addition of more acid.

At the isoelectric point no protein is ionized and z being zero, the whole term

$$\sqrt{4y^2 + 4yz + z^2} - \sqrt{4y^2 + 4yz}$$

becomes zero. Hence at the isoelectric point the observed osmotic pressure is purely that due to the protein, which is very low on account of the high molecular weight of gelatin.

When little acid, *e. g.*, HCl, is added to the solution of isoelectric gelatin, gelatin chloride is formed and some free acid remains, due to hydrolytic dissociation. Hence both z (the concentration of Cl ions in combination with protein) and y (the Cl ions of the free HCl existing through hydrolysis) increase, but z increases at first more rapidly than y and hence the excess of concentration of ions inside over that of ions outside increases until the greater part of protein is transformed into protein chloride, when the excess of crystalloidal ions inside over those outside reaches a maximum. From then on z increases comparatively little while y increases considerably with further addition of acid, so that z becomes negligible in comparison with y. This explains why the Donnan correction becomes zero again when enough acid is added, and why the observed osmotic pressure becomes as low again as at the isoelectric point.

In the same way it can be shown why the addition of salt has only a depressing effect on the osmotic pressure. Let us assume that there is inside the bag a gelatin chloride solution of p_H 3.0 to which NaCl is added. z (the concentration of Cl ions in combination with the gelatin) will not increase with the addition of salt, while y (the concentration of the Cl ions *not* in combination with gelatin) will increase. Hence with the increase in the concentration of the salt the value of

$$\sqrt{4y^2 + 4yz + z^2} - \sqrt{4y^2 + 4yz}$$

will become smaller, finally approaching zero.

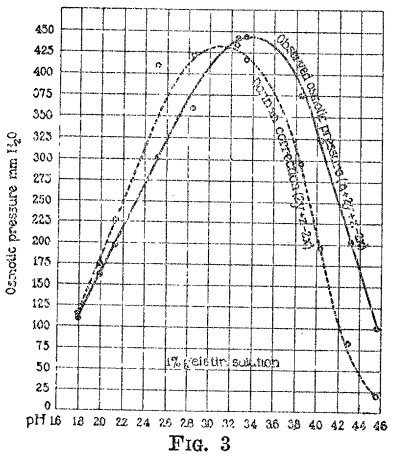

FIG. 3

When another salt than a chloride, *e. g.*, $NaNO_3$, is added to a solution of gelatin chloride, we may assume that the gelatin in solution is gelatin nitrate.

Fig. 3 gives a comparison of the curves for the observed osmotic pressure and for the Donnan correction. Both curves rise in a parallel way from the isoelectric point reaching a maximum which is 450 mm. H_2O pressure in the case of the observed osmotic pressure and slightly lower in the case of the Donnan correction. The observed osmotic pressure should be higher than the Donnan correction by the osmotic pressure due to the protein solution itself. A difference exists in the values between p_H 4.6 and 3.2 but disappears later, and this difference is in all probability the expression of value *a, i. e.*, the osmotic pressure due to the protein itself. The disappearance of this difference at p_H below 3.2 is probably due to the fact that an error of one unit in the second decimal of the p_H causes a considerable error in the calculations of z which increases when the p_H is too low.

Fig. 3 shows that when we correct the observed osmotic pressure for the Donnan effect it follows that the influence of the p_H of the acid on the osmotic pressure is entirely or practically entirely due to the excess of the concentration of crystalloidal ions inside the membrane over that outside and that this excess is caused by the Donnan equilibrium. The osmotic pressure of the protein itself is either not altered at all by the addition of acid or if it is altered the effect is too small to be noticeable. There is then nothing left for the "dispersion theory" or for any other of the colloidal speculations to explain. These results were confirmed for crystalline egg albumin and casein by the writer and for edestin by Hitchcock. We now understand why only the valency and not the nature of the ion plays a rôle in the osmotic pressure of protein solutions. The equilibrium equation is one of the second degree when the ion with which the protein is in combination is monovalent while it is of the third degree when the ion is divalent. Only the valency of the ion and not its nature enters into the Donnan equation.

We can therefore summarize these results by

stating that the so-called colloidal behavior of protein solutions, as far as osmotic pressure is concerned, is merely the result of an equilibrium condition of classical chemistry which results in an excess of the concentration of crystalloidal ions inside the protein solution over that of an outside aqueous solution, when the two solutions are separated by a membrane which is permeable to crystalloidal ions but impermeable to protein ions. The colloidal behavior of proteins depends therefore entirely on the relative non-diffusibility of protein ions through membranes which are easily permeable to crystalloidal ions. Since the majority of membranes in plants and animals belong to this class, it can easily be surmised how great a rôle the proteins must play in the regulation of osmotic pressure in the body.

VI

It remains to show briefly why swelling and viscosity of protein solutions are affected in a similar way by electrolytes as is the osmotic pressure. The answer is that we are dealing in both cases with the same fundamental property, namely, osmotic pressure.

In 1910 Procter made the ingenious suggestion that the swelling of gelatin might be an osmotic phenomenon and in subsequent papers he and J. A. Wilson put this theory on a quantitative basis by deriving it from the Donnan equilibrium. They showed that the swelling of a solid gel of gelatin in hydrochloric acid can be explained quantitatively on the basis of the Donnan equilibrium on the assumption that there exists an excess of concentration of crystalloidal ions inside (in this case H and Cl) over the concentration of the same ions outside, and that the excess of osmotic pressure inside the gel over that outside due to this Donnan effect accounts for that share of the swelling which is caused by the influence of the acid. The agreement of their calculated values with the observed values is excellent. The writer is inclined to consider Procter's theory of swelling and the proof of this theory by Procter and J. A. Wilson as the most brilliant contribution to the theory of colloidal behavior next in importance only to Donnan's theory of membrane equilibria. There was only one detail left by these authors, namely, to prove the existence of membrane potentials between the gel and the surrounding aqueous solution at equilibrium. The writer was able to fill this gap and to show that the observed P.D. between gel and surrounding aqueous solution can be calculated with a fair degree of accuracy from the value p_H inside minus p_H outside with the aid of Nernst's logarithmic formula.

VII

It may seem strange that the influence of electrolytes on the viscosity of certain protein solutions should be explained in the same way, but this seems to be the case. According to Einstein's formula, the viscosity of an aqueous protein solution is a linear function of the relative volume of the solute occupied in the solution, as expressed in the formula

$$\eta = \eta_0(1 + 2.5\varphi)$$

where η is the viscosity of the solution, η_0 that of pure water, and φ the proportion of the volume of the solute to that of the solution. If, therefore, the addition of little acid to a 1 per cent. solution of isoelectric gelatin increases the viscosity of the solution until a maximum is reached and if the addition of more acid depresses the viscosity again, it follows that the addition of acid changes the relative volume occupied by the gelatin in water. This is only possible by water being absorbed by the protein and the question is how to account for this absorption of water by the protein under the influence of acid. Pauli assumed that the ionized protein surrounds itself with a jacket of water which is lacking in the non-ionized protein. If this were true, all the proteins and amino-acids should show such an influence of acid on the viscosity of their solutions. The writer found that no such influence exists in the case of amino-acids and at least one protein, namely, crystalline egg albumin; if Pauli's assumption were correct, there is no reason why crystalline egg albumin should not show the same influence of acid on viscosity which is found in the case of gelatin. The difference between gelatin and crystalline egg albumin is that the former sets to a solid gel

if the temperature is not too high while the latter does not. The formation of a continuous gel in the gelatin solution is preceded by the formation of submicroscopic aggregates which occlude water and which are capable of swelling and these aggregates or precursors of the continuous gel increase in size and number on standing. To test this idea the writer made experiments with suspensions of powdered gelatin in water and found that such suspensions of powdered gelatin had a much higher viscosity than a freshly prepared solution of gelatin. This was to be expected if the influence of acid on the viscosity of proteins is due to the swelling of submicroscopic particles of gel. It harmonizes with this fact that the viscosity of solutions of crystalline egg albumin is of a low order of magnitude, which was to be expected if solutions of crystalline egg albumin contain few or no micellæ. It was found, moreover, that the viscosity of suspensions of powdered gelatin increased under the influence of acid or alkali in the same way as did the swelling of jellies or the osmotic pressure of protein solutions. The viscosities were measured at 20° C. When the suspension of powdered gelatin was melted, it was found upon rapid cooling to 20° C. that the viscosity was considerably lower and that the influence of acid had almost disappeared. By these and a number of similar experiments it was possible to prove that the similarity between the influence of electrolytes on the viscosity of gelatin solution and the influence of electrolytes on osmotic pressure is due to the fact that the influence on viscosity in such cases is in reality an influence on the swelling of submicroscopic protein particles. This proof was made complete by showing that there exists a Donnan equilibrium between powdered particles of gelatin and a surrounding weak gelatin solution.

VIII

It may not be amiss to illustrate by way of an example why it is that the neglect of measuring the hydrogen ion concentration of protein solutions necessarily leads into errors. In a paper published in 1921 by Kuhn,[3] it was intended to show that different acids of the same valency have different effects on the swelling of gelatin. In order to furnish such a proof it is necessary to start with isoelectric gelatin and to compare the effect of different acids on the swelling of this isoelectric gelatin at the same hydrogen ion concentration of the gel, since only in that case have the gels the same concentration of gelatin ions. Instead of starting with isoelectric gelatin or gelatin of a measured p_H, Kuhn failed to measure the p_H of his gelatin, though it makes quite a difference whether acid is added to isoelectric gelatin or to gelatin at another p_H. Further, instead of measuring the p_H of the gel with the hydrogen electrode, Kuhn calculated the hydrogen ion concentrations from Kohlrausch's tables as if acid had been added to water free from gelatin and as if the presence of the protein did not alter the hydrogen ion concentration. Our titration curves, however, show that when acid is added to isoelectric gelatin the hydrogen ion concentration is less than when acid is added to water free from protein. And finally, on account of the Donnan equilibrium the p_H inside and outside the gel are entirely different; yet no mention is made of the Donnan equilibrium in the paper referred to. The hydrogen ion concentrations of protein solutions which were considered as equal by Kuhn were on account of all these errors entirely different, and it is quite natural that Kuhn came to the conclusion that different monobasic acids have different effects on swelling, since it would have been a miracle if with his faulty methods he had ever compared two acids of the same p_H. The same criticism applies to all the older experiments on the influence of electrolytes on swelling in which the authors reached the conclusions that different anions of the same valency have different effects on swelling (Hofmeister series). In all these experiments the authors failed to measure the p_H of their gels and erroneously attributed effects due to differences of the p_H of the gels to the difference in the nature of the anion.

IX

We therefore come to the conclusion that the chemistry of proteins does not differ from the chemistry of crystalloids, and that proteins combine stoichiometrically with acids and alkalies forming protein salts which dissociate electrolytically. The enormously large protein ions and molecules can not diffuse freely through gels or many membranes which are easily permeable to small crystalloidal ions.

[3] Kuhn, A.: *Kolloidchem. Beihefte,* 1921, xiv, 147.

This fact leads, under proper conditions, to an unequal distribution of the diffusible crystalloidal ions between a protein solution and an outside aqueous solution; or between a protein gel and an aqueous solution. In this distribution the total concentration of crystalloidal ions is always greater inside the protein solution or inside a gel than in the surrounding aqueous solution. This is the cause of the colloidal behavior of protein solutions and protein gels. Measurements of membrane potentials have shown that this excess of the concentration of crystalloidal ions inside over the concentration of the crystalloidal ions outside the protein solution or the gel, and consequently all the effects of electrolytes on osmotic pressure, swelling and viscosity of proteins, can be calculated with a satisfactory degree of accuracy from Donnan's equilibrium equation, which is not an empirical but a rationalistic mathematical formula. We can therefore state that it is possible to explain the colloidal behavior of proteins quantitatively on the basis of a rationalistic mathematical formula. What appeared at first as a new chemistry, the so-called colloid chemistry, now seems to have been only an overlooked equilibrium condition of classical chemistry; at least as far as the proteins are concerned. The oversight was due to two facts, first, to the failure of colloid chemists to measure the hydrogen ion concentration of their solutions, which happens to be the chief variable in the case, and second, to their neglect of measuring and taking into consideration the membrane potentials of protein solutions and protein gels, which furnish the proof that the theory of membrane equilibria must be used to explain the colloidal behavior of proteins.

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THE AWARD OF THE HENRY DRAPER MEDAL

THE Henry Draper medal for 1921, awarded by the National Academy of Sciences to Professor Henry Norris Russell, professor of astronomy at Princeton University, was presented to him by Dr. C. G. Abbot, assistant director of the Smithsonian Institution at the annual dinner in New York City on November 15. Dr. Abbot spoke as follows:

The brilliant and penetrating insight of Dr. Henry Norris Russell, of Princeton University, has led in recent years to a development of astronomy so rapid that it has proved thus far impossible to publish really up-to-date text-books on the subject. Before the manuscript of a text on astronomy can be prepared, much less carried through the press, new knowledge renders the treatment stale.

Dr. Russell has made basic contributions to the great problem of stellar evolution. He saw clearly that the brightness of a star as we see it depends on several factors. First, there is the intrinsic brightness of the star as a source of light. What the tallow candle is to the electric arc, so one star may be to another in the brightness of its shining surface. Secondly, the total amount of light which a star sends out depends upon its diameter. Quite recently it has been shown, for instance, that the star Alpha Orionis is three hundred times the diameter of the sun, and accordingly its cross-sectional area is ninety thousand times the cross-sectional area of the sun. Hence, if they were of equal surface brightness, the star Alpha Orionis would send out ninety thousand times as much light as the sun. In the third place, the brightness of the star depends upon its distance from the earth and falls off as the square of that distance. Thus, the sun, which is so near that it takes light eight minutes to come from it, being about two hundred thousand times as near as the next nearest star which takes light three or four years to reach the earth will appear forty million times brighter on that account.

With these conditions in mind, Dr. Russell, in collaboration with Dr. Hinks, of England, began by the application of a new photographic method of determining the distance of stars, and in 1910 published the results showing the approximate distance of 55 stars. With this and other such information which had been laboriously acquired by others, he was able to show that the red stars evidently must fall into two classes: one class sending out very much more light than our sun, and another sending out very much less, and that between these two very widely separated extremes there are no red stars intervening.

Going on, he applied the, until then little used, knowledge of the eclipsing variable stars with the